

CLAIMS

What is claimed is:

1 1. A substantially complete ribozyme library comprising a collection of
2 adeno-associated virus (AAV), retroviral, or Epstein-Barr virus (EBV) vectors, or a
3 collection of retroviral vectors containing nucleic acids encoding hairpin ribozymes in
4 expression cassettes wherein said collection of AAV, retroviral, or EBV vectors contains
5 nucleic acids encoding on average about 90% or more of all possible hairpin ribozyme
6 binding sequences having eight or more randomized nucleotides.

1 2. The ribozyme library of claim 1, wherein said collection of vectors
2 contains nucleic acids encoding about 95% or more of all possible hairpin ribozyme binding
3 sequences.

1 3. The ribozyme library of claim 1, wherein said collection of vectors
2 contains nucleic acids encoding about 95% or more of all possible hairpin ribozyme binding
3 sequences having 9 or more randomized nucleotides.

1 4. The ribozyme library of claim 1, wherein said collection of vectors
2 contains nucleic acids encoding about 95% or more of all possible hairpin ribozyme binding
3 sequences having 12 randomized nucleotides.

1 5. The ribozyme library of claim 1, wherein said nucleic acids are plasmids.

1 6. The ribozyme library of claim 1, wherein said library contains no toxic
2 ribozymes.

1 7. The ribozyme library of claim 1, wherein said collection of vectors is a
2 collection of AAV vectors.

1 8. The ribozyme library of claim 7, wherein said nucleic acids comprise a pair
2 of inverted terminal repeats (ITRs) of adeno-associated viral genome.

1 9. The ribozyme library of claim 1, wherein said nucleic acids comprise a
2 selectable marker.

10. The ribozyme library of claim 9, wherein said selectable marker is selected from the group consisting of Neo^r, and Hygro^r.

11. The ribozyme library of claim 10, wherein said selectable marker is operably linked to an SV40 promoter.

12. The ribozyme library of claim 1, wherein the ribozyme-encoding nucleic acid is operably linked to a tRNA promoter.

13. The ribozyme library of claim 1, wherein the ribozyme-encoding nucleic acid is operably linked to a promoter selected from the group consisting of tRNA^{Val}, tRNA^{Ser}, and PGK.

14. A substantially complete ribozyme gene library comprising a collection of plasmids wherein members of said collection encode a retroviral, adeno-associated virus (AAV), or Epstein Barr virus (EBV) vector containing a ribozyme-encoding nucleic acid and said collection of plasmids encodes on average about 90% or more of all possible hairpin ribozyme binding sequences having eight or more randomized nucleotides.

15. The ribozyme gene library of claim 14, wherein said collection of plasmids encodes on average about 95% or more of all possible hairpin ribozyme binding sequences.

16. The ribozyme gene library of claim 14, wherein said collection of plasmids encodes on average about 95% or more of all possible hairpin ribozyme binding sequences having 9 or more randomized nucleotides.

17. The ribozyme gene library of claim 14, wherein said library contains essentially no toxic ribozymes.

18. The ribozyme gene library of claim 14, wherein members of said collection encode an AAV vector.

19. The ribozyme gene library of claim 18, wherein said nucleic acids comprise a pair of inverted terminal repeats (ITRs) of adeno-associated viral genome.

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1 20. The ribozyme gene library of claim 14, wherein said plasmids contain a
2 selectable marker.

1 21. The ribozyme gene library of claim 20, wherein said selectable marker is
2 selected from the group consisting of Neo^r, and Hygro^r.

1 22. The ribozyme gene library of claim 21, wherein said selectable marker is
2 operably linked to an SV40 promoter.

1 23. The ribozyme gene library of claim 14, wherein the ribozyme-encoding
2 nucleic acid is operably linked to a tRNA promoter.

1 24. The ribozyme gene library of claim 14, wherein the ribozyme-encoding
2 nucleic acid is operably linked to a promoter selected from the group consisting of tRNA^{Val},
3 tRNA^{Ser}, and PGK.

1 25. A method of selecting a ribozyme that specifically binds and cleaves a
2 nucleic acid target, said method comprising:

3 i) transfecting a population of cells with a substantially complete
4 hairpin ribozyme library comprising a collection of adeno-associated virus (AAV), retroviral,
5 or Epstein Barr virus (EBV) vectors containing nucleic acids encoding hairpin ribozymes in
6 expression cassettes wherein said collection of AAV, retroviral, or EBV vectors contains
7 nucleic acids encoding on average about 90% or more of all possible hairpin ribozyme
8 binding sequences having eight or more randomized nucleotides;

9 ii) detecting a phenotypic difference between a transfected cell
10 that expresses at least one hairpin ribozyme encoded by said library and a control cell lacking
11 an active members of said ribozyme library, wherein said phenotypic difference is a
12 consequence of cleavage of said target; and

13 iii) recovering a ribozyme associated with said phenotypic
14 difference.

1 26. The method of claim 25, wherein said transfecting produces a population
2 of cells stably transfected with an expression cassette encoding a hairpin ribozyme.

1 27. The method of claim 25, wherein said hairpin ribozyme is constitutively
2 expressed.

1 28. The method of claim 25, wherein said recovering comprises isolating a
2 multiplicity of ribozymes to produce a targeted ribozyme library.

1 29. The method of claim 28, further comprising
2 iv) transfecting a population of cells with said targeted ribozyme
3 library;
4 v) detecting a phenotypic difference between a transfected cell
5 that expresses at least one hairpin ribozyme encoded by said targeted ribozyme library and a
6 control cell lacking an active member of said ribozyme library, wherein said phenotypic
7 difference is a consequence of cleavage of said target; and
8 vi) recovering a ribozyme associated with said phenotypic
9 difference.

1 30. The method of claim 25, wherein said recovering comprises isolating and
2 sequencing the binding site of said ribozyme.

1 31. The method of claim 30, further comprising providing a probe that
2 hybridizes to the nucleic acid specifically bound by said ribozyme.

1 32. The method of claim 31, wherein said probe is labeled.

1 33. The method of claim 25, wherein phenotypic difference is a difference in
2 transcription or expression of a reporter gene or cDNA.

1 34. The method of claim 25, wherein phenotypic difference is the ability of a
2 cell to grow on soft agar.

1 35. The method of claim 25, wherein phenotypic difference is the ability of a
2 cell to differentiate.

1 36. The method of claim 35, wherein said ability to differentiate is identified
2 by the adherence of the cell to a surface in culture.

1 37. The method of claim 25, wherein said phenotypic difference is resistance
2 to a drug.

1 38. The method of claim 37, wherein said drug is selected from the group
2 consisting of cisplatin, doxorubicin, taxol, camptothecin, daunorubicin, and methotrexate.

1 39. The method of claim 25, wherein said phenotypic difference is a change
2 in the expression level of a reporter gene linked to a gene whose regulation it is desired to
3 alter.

1 40. The method of claim 25, wherein said collection of AAV, retroviral, or
2 EBV vectors contains nucleic acids encoding on average about 95% or more of all possible
3 hairpin ribozyme binding sequences.

1 41. The method of claim 25, wherein said collection of AAV, retroviral, or
2 EBV vectors contains nucleic acids encoding on average about 90% or more of all possible
3 hairpin ribozyme binding sequences having 9 or more randomized nucleotides.

1 42. The method of claim 25, wherein said nucleic acids are plasmids.

1 43. The method of claim 25, wherein said library contains no toxic ribozymes.

1 44. The method of claim 25, wherein said collection of vectors is a collection
2 of AAV vectors.

1 45. The method of claim 44, wherein said nucleic acids comprise a pair of
2 inverted terminal repeats (ITRs) of adeno-associated viral genome.

1 46. The method of claim 25, wherein said nucleic acids comprise a selectable
2 marker.

1 47. The method of claim 46, wherein said selectable marker is selected from
2 the group consisting of Neo^r and Hygro^r.

1 48. The method of claim 47, wherein said selectable marker is operably
2 linked to an SV40 promoter.

1 49. The method of claim 25, wherein the ribozyme-encoding nucleic acid is
2 operably linked to a tRNA promoter.

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1 50. The method of claim 25, wherein the ribozyme-encoding nucleic acid is
2 operably linked to a promoter selected from the group consisting of tRNA^{Val}, tRNA^{Ser}, and
3 PGK.

1 51. A method of identifying a gene or mRNA altered expression of which
2 results in alteration of a detectable phenotypic character, said method comprising:
3 i) stably transfecting a population of cells with a hairpin ribozyme
4 library comprising a collection of adeno-associated virus (AAV) vectors containing nucleic
5 acids encoding hairpin ribozymes in expression cassettes;
6 ii) detecting a phenotypic difference between a transfected cell
7 that expresses said hairpin ribozyme and a control cell lacking an active form of said hairpin
8 ribozyme;
9 iii) recovering a ribozyme associated with said phenotypic
10 difference; and
11 iv) sequencing the binding site sequence of the recovered ribozyme
12 to identify the nucleic acid to which it bound.

1 52. The method of claim 51, wherein said hairpin ribozyme is constitutively
2 expressed.

1 53. The method of claim 51, wherein said ribozyme library is a substantially
2 complete ribozyme library.

1 54. The method of claim 51, wherein said ribozyme library is a targeted
2 ribozyme library.

1 55. The method of claim 51, wherein said recovering comprises reverse
2 transcribing and amplifying the nucleic acid comprising said ribozyme..

1 56. The method of claim 55, further comprising providing a probe that
2 hybridizes to the nucleic acid specifically bound by said ribozyme.

1 57. The method of claim 56, wherein said probe is labeled.

1 58. The method of claim 51, wherein said phenotypic difference is a
2 difference in transcription or expression of a reporter gene or cDNA.

1 59. The method of claim 51, wherein said phenotypic difference is the ability
2 of a cell to grow on soft agar.

1 60. The method of claim 51, wherein said phenotypic difference is the ability
2 of a cell to differentiate.

1 61. The method of claim 60, wherein said ability to differentiate is identified
2 by the adherence of the cell to a surface in culture.

1 62. The method of claim 51, wherein phenotypic difference is resistance to a
2 drug.

1 63. The method of claim 62, wherein said drug is selected from the group
2 consisting of cisplatin, doxorubicin, taxol, camptothecin, daunorubicin, and methotrexate.

1 64. The method of claim 51, wherein said phenotypic difference is a change
2 in the expression level of a reporter gene linked to a gene whose regulation it is desired to
3 alter.

1 65. A method of producing a cell line having altered expression of a gene said
2 method comprising stably transfecting a cell with a vector encoding a hairpin ribozyme
3 wherein said hairpin ribozyme is identified according to the method of claim 25.

1 66. A population of mammalian cells containing a substantially complete
2 ribozyme library comprising a collection of adeno-associated virus (AAV), retrovirus, or
3 Epstein Barr virus (EBV) vectors containing nucleic acids encoding hairpin ribozymes in
4 expression cassettes wherein said collection of AAV, retroviral, or EBV vectors contains
5 nucleic acids encoding on average about 90% or more of all possible hairpin ribozyme
6 binding sequences having eight or more randomized nucleotides.

1 67. The ribozyme library of claim 66, wherein said collection of AAV,
2 retroviral, or EBV vectors contains nucleic acids encoding about 95% or more of all possible
3 hairpin ribozyme binding sequences.

1 68. The ribozyme library of claim 66, wherein said collection of AAV,
2 retroviral, or EBV vectors contains nucleic acids encoding about 95% or more of all possible
3 hairpin ribozyme binding sequences having 9 or more randomized nucleotides.

1 69. The ribozyme library of claim 66, wherein said collection of AAV,
2 retroviral, or EBV vectors contains nucleic acids encoding about 95% or more of all possible
3 hairpin ribozyme binding sequences having 12 randomized nucleotides.

1 70. A kit comprising one or more containers containing
2 a substantially complete ribozyme library comprising a collection of
3 adeno-associated virus (AAV), retrovirus, or Epstein Barr virus (EBV) vectors containing
4 nucleic acids encoding hairpin ribozymes in expression cassettes wherein said collection of
5 AAV, retroviral, or EBV vectors contains nucleic acids encoding on average about 90% or
6 more of all possible hairpin ribozyme binding sequences having eight or more randomized
7 nucleotides; or
8 a substantially complete ribozyme gene library comprising a collection
9 of plasmids wherein members of said collection encode a retroviral, adeno-associated virus
10 (AAV), or Epstein Barr virus (EBV) vector containing a ribozyme-encoding nucleic acid and
11 said collection of plasmids encodes on average about 90% or more of all possible hairpin
12 ribozyme binding sequences having eight or more randomized nucleotides.